Human Dermal Fibroblasts Present Tetanus Toxoid Antigen to Antigen-specific T Cell Clones

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Abstract

Cultured human dermal fibroblasts treated with immune interferon express HLA-DR antigens. We report here that DR-positive fibroblasts present tetanus toxoid (TT) to autologous TT-specific monoclonal helper T cells vigorously depleted of monocytes by passage over Sephadex G10 columns followed by treatment with the monoclonal antibodies (mAb) OKM1 and Leu M1 plus complement. The extent of T cell proliferation in response to TT presented by DR-positive fibroblasts was similar to that elicited using monocytes as antigen-presenting cells. The proliferative response was TT dependent, antigen specific, depended upon DR expression by fibroblasts, appeared MHC restricted, and was completely blocked by mouse mAb to HLA-DR but not by mAb to HLA-A,B, or DQ. DR-positive fibroblasts pulsed with TT were similarly effective in antigen presentation. In summary, immune interferon-stimulated human dermal fibroblasts can substitute for classical antigen-presenting cells in antigen-specific proliferative responses. Since fibroblasts are a ubiquitous cell type in the body, they may play a significant role in the immunobiology of the host.

Introduction

The induction of T lymphocyte responses requires antigen presentation by accessory cells (AC).[1] Macrophages, as well as epidermal Langerhan's cells (1–3), dendritic cells of lymphoid organs (4, 5), some lymphoblastoid B cell lines (6–8), vascular endothelial cells (3, 9), and brain astrocytes (10), are capable of AC function and antigen presentation. All of these cells display surface Class II major histocompatibility complex (MHC) antigens (HLA-DR, DP, DQ, or Ia antigens), which are recognized by the responding T cell. Knowledge of the T cell repertoire has indicated that expression of Ia is necessary for antigen presentation, since T helper cells see antigens only in the context of the polymorphic determinants of Ia molecules (reviewed in reference 11). The range of cells capable of presenting antigen to T cells is not known.

Recently, we have shown that human class II antigens can be induced on human dermal fibroblasts that are exposed to immune interferon (IFN-γ) (12, 13). We now report that such HLA-DR-positive fibroblasts are capable of presenting tetanus toxoid (TT) antigen to human antigen-specific monoclonal helper T cells. Thus, IFN-γ-stimulated fibroblasts can substitute for classical antigen presenting cells (APC) in antigen-specific proliferative responses. Since fibroblasts are a ubiquitous cell type in the body, they may play a significant role in the immunobiology of the host.

Methods

Antigens. TT and diphtheria toxoid (DT) were obtained from the Massachusetts Biological Laboratories, Boston, MA. Monilia was obtained from Hollister-Stier Laboratories, Media, PA. All antigens were dialyzed extensively against phosphate-buffered saline (PBS) before use.

Preparation of T cell clones. TT-specific lymphocyte clones were produced by limiting dilution in a manner described by Lamb et al. (14) for influenza specific clones. Briefly, peripheral blood mononuclear cells (PBMC) were stimulated with TT (30 μg/ml, 10^6 cells/ml) for 6 d. Dividing cells were then enriched by centrifugation over a Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) discontinuous gradient at 1,500 rpm for 45 min. Cells at the 30–50% interface were cultured in RPMI 1640 (M.A. Biologicals, Bethesda, MD) at limiting dilution (0.3 cell/well) with autologous irradiated PBMC (10^5/well) added every 7 d. These clones have been maintained in culture for >1 yr by repeated stimulation with TT and autologous PBMC with interleukin-2 (IL-2)-containing supernatants, in RPMI 1640 containing 10% fetal calf serum (FCS) (Sterile Systems, Inc., Logan, UT).

Two clones were used. G8 is a TT-specific clone restricted by HLA-DR5 (or HLA-DR5 linked) antigens. Clone F6 is a TT-specific clone restricted by HLA-DR3 (or HLA-DR3 linked) antigens (15). Clones were used in fibroblast cultures 14–28 d after the last addition of irradiated feeder cells.

Interleukin-2-containing supernatants. PBMC were obtained from donors previously screened for their capacity to generate high-activity IL-2. PBMC were depleted of monocytes by adherence to plastic Petri dishes and the nonadherent cells were irradiated (1,000 rad). Cells were suspended at 10^6 cells/ml in RPMI 1640, with 2% AB positive serum containing phytohemagglutinin (1 μg/ml, Wellcome Laboratories, Research Triangle Park, NC), and harvested after 48 h.

Monocyte depletion of T cell clones. Cloned T cells cultured for 21 d with IL-2-containing supernatants in the absence of added irradiated PBMC were passed over columns of Sephadex G10 according to the method of Ly et al. (16). Nonadherent cells were then incubated with OKM1 and Leu M1 for 45 min at 0°C. Baby rabbit complement (Pel-

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1. *Abbreviations used in this paper:* AC, accessory cell(s); APC, antigen presenting cells; DT, diphtheria toxoid; EBV, Epstein-Barr virus; FCS, fetal calf serum; IL-2, interleukin-2; IFN-γ, immune interferon; mAb, monoclonal antibody(s); MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell(s); TT, tetanus toxoid.

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Freeze Biologicals, Rogers, AR) was added (final dilution 1:2). Clones were then washed three times and then cultured with irradiated APC with and without antigen.

Fibroblast cultures. 3-mm skin punch biopsies obtained from donors were cut into 1-mm sections and cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) containing 10% heat-inactivated FCS. After the skin fibroblasts had grown out of the explants and reached confluency, they were trypsinized and transferred to tissue culture flasks. These cells were maintained in culture in Medium 199 (M.A. Bioproducts, Walkersville, MD), 15% FCS, and split every 1–2 wk as they reached confluency. All experiments were performed with skin fibroblasts taken after 10 to 20 culture passages. Fibroblast cultures consisted of uniform bipolar spindle-shaped cells. Untreated cells were uniformly negative for HLA-DR expression by radioimmunoassay or by cell sorter analysis (see below).

Interferon treatment. Fibroblasts were plated in microtiter plates (NUNC, Roskilde, Denmark), $5–10 \times 10^3$/well, and grown to confluence. Recombinant IFN-γ, 100 U/ml, was added to the fibroblasts for 96 h, then washed 4–5 times before use as previously described (12). In some experiments, recombinant human IFN-γ was obtained from Genentech, Inc., South San Francisco, CA.

Proliferation assays. 4×10^4 cloned T cells were added to triplicate wells of 96-well flat-bottomed microtiter plates containing fibroblasts grown to confluence ($\sim 2 \times 10^4$ fibroblasts/well) and gamma irradiated (3,400 rad). In parallel cultures, irradiated (7,500 rad) Epstein-Barr virus (EBV) transformed B cells (4×10^4 cells/well), or irradiated (2,500 rad) adherent monocytes (2×10^4 cells/well), were added to T cells as APC. Thymidine incorporation was assessed by pulsing with 0.8 μCi methyl [^{3}H]thymidine (New England Nuclear, Boston, MA) during the last 18 h of the 96-h culture.

Monoclonal antibodies (mAb). LB3.1 (17), an IgG_{2b} antibody to human HLA-DR framework antigens, and W6/32 (18), an IgG_{2a} antibody to human HLA-A,B framework antigens, were the generous gifts of Jack L. Strominger, Dana-Farber Cancer Institute, Boston, MA. ST2-59, an IgG_{2a} antibody to human HLA-DR framework antigens, was the generous gift of Dr. Soo Young Yang, Dana-Farber Cancer Institute. Leu 10, an antibody to DQ antigens (19), was purchased from Becton-Dickinson & Co., Sunnyvale, CA, and UPC-10, a mouse myeloma protein of IgG_{2a} subclass, was purchased from Walgene R & D Laboratories, Arcadia, CA. OKM1 (20) was purchased from Ortho Diagnostic Systems Inc., Raritan, NJ, and Leu M1 (21) was purchased from Becton-Dickinson & Co.

HLA-DR typing. Tissue typing was performed on peripheral blood leukocytes by the HLA laboratory at the Dana Farber Cancer Institute. HLA-DR on fibroblasts was analyzed by cytotoxicity with standard typing sera or with mAb to DR antigens. In other experiments presence of DR antigens on fibroblasts was assessed by mAb binding quantitated with radioactive second antibody or by cell sorter analysis of trypsinized fibroblasts using fluorescent second antibody as previously described (22). Cultured fibroblasts treated with IFN-γ for 96 h were uniformly positive for expression of HLA-DR, DP, and DQ, as reported elsewhere (12, 13).

Results

Antigen presentation by fibroblasts to T cell clones. Monoclonal T cells specific for the antigen TT were established as previously described (14, 15). These long-term cloned T cells have been cultured by repeated stimulation (every 7–10 d) with TT in the presence of irradiated autologous PBMC with IL-2-containing supernatants, and were shown to proliferate on exposure to TT in the context of specific HLA-DR antigens on monocytes. Table I shows that significant proliferation of cloned T cells occurred in the presence of TT antigen and autologous monocytes, or with TT and autologous fibroblasts that were pretreated with IFN-γ (lines 3–6). Whenever examined in the same experiment, the extent of T cell proliferation to IFN-γ-treated fibroblasts plus TT was similar to that seen in the presence of autologous monocytes (Table I). The proliferation of both clones F6 and G8 was antigen- and AC-dependent, as it did not occur in the presence of TT alone (Table I, line 2), nor in the presence of IFN-γ-treated fibroblasts alone (Table I, line 5). Untreated fibroblasts failed to present TT to the T cell clones.

Although cloned T cells were maintained without the addition of feeder cells for 14–28 d before testing, and did not proliferate to antigen without the addition of accessory cells (Table I, line 1), it was imperative to demonstrate that the capacity of IFN-γ-treated fibroblasts to stimulate antigen-specific proliferation of the T cell clones was not dependent on the presence of residual monocytes contaminating the T cell clones. Cloned T cells were therefore passed over Sephadex G10, treated with OKM1 and Leu M1 plus complement, then examined for their proliferative response to TT. Such treatment, when applied to PBMC, results in total loss of proliferation to

Table I. IFN-γ-treated Fibroblasts Present Antigen to Autologous TT-specific T Cell Clones

		CPM of [^{3}H]thymidine incorporated per culture±SD			
APC	Antigen	Exp. 1: clone G8	Exp. 2: clone F6	Exp. 3: clone F6	Exp. 4: clone F6
None	—	103±24	357±164	117±27	176±32
None	TT*	139±111	118±56	87±16	187±51
Monocytes	—	221±66	999±269	332±84	749±180
Monocytes	TT	30,900±1,787	17,933±1,249	15,818±1,219	10,775±1,265
Fibroblasts IFN-γ‡	—	181±99	281±124	381±51	186±93
Fibroblasts IFN-γ	TT	37,374±4,388	10,067±1,276	7,713±1,605	9,643±1,257
Fibroblasts	—	133±27	907±99	217±52	375±67
Fibroblasts	TT	170±68	425±124	402±27	866±130

Clones were maintained without irradiated PBMC feeder cells for 14–28 d before performing the experiments.
* TT was added at 30 μg/ml. ‡ Fibroblasts IFN-γ: gamma interferon-treated fibroblasts. Thymidine incorporation of fibroblast cultures alone was 194±119 cpm in exp. 1, 436±189 in exp. 2, 497±337 in exp. 3, and 159±74 in exp. 4. TdH^3 incorporation of monocyte cultures was always <200 cpm.

Table II. Passage of Clones Over Sephadex G10 and Lysis with OKM1 and Leu M1 Does Not Affect Their Proliferative Response to TT Antigen Presented by Autologous IFN-γ-treated Fibroblasts

		[³H]Thymidine incorporated per culture ±SD			
		Monocyte-depleted clones		Untreated clones	
Clone	APC	—	TT	—	TT
G8	—	189±113	127±50	137±27	310±29
	Fibroblasts IFN-γ	347±94	9,516±1,542	214±137	10,291±709
F6	—	80±20	61±10	169±21	150±36
	Fibroblasts IFN-γ	142±23	3,709±387	175±23	3,608±338

Values represent mean±SD of triplicate cultures. Culture conditions were as described in Table I. Fibroblasts alone incorporated <200 cpm.

antigens (tetanus and DT), and is reversed by the addition of autologous monocytes. Examination of T cell clones treated in this manner revealed <0.1% monocytes, as evidenced by esterase staining and by fluorescent staining using OKM1 followed by goat anti-mouse IgG conjugated to fluorescein isothiocyanate. Table II shows that these monocyte-depleted cloned T cells proliferated vigorously to TT antigen in the presence of autologous IFN-γ-treated fibroblasts. The proliferation of T cell clones vigorously depleted of monocytes was equivalent to that of untreated T cell clones (Table II). In both cases, T cell proliferation in the presence of TT and IFN-γ-treated fibroblasts was equivalent to that seen with TT and monocytes (data not shown).

Antigen specificity of fibroblast presentation. Antigen presentation by IFN-γ-treated fibroblasts to T cell clones was antigen specific. Table III shows that IFN-γ-treated autologous fibroblasts, like autologous monocytes, induce TT-specific T cell clones to proliferate only in the presence of TT antigen, and not in the presence of other antigens such as DT and monilia.

IFN-γ-treated fibroblasts pulsed with TT antigen for 20 h stimulated T cell proliferation as effectively as when soluble TT was added throughout the culture period (Table IV). However, fibroblasts that were not pretreated with IFN-γ, but which were pulsed with TT, were not effective in stimulating proliferation.

Role of HLA-DR antigens. The role of HLA-DR antigens on IFN-γ-pretreated fibroblasts in T cell proliferation in response to antigen was examined. A panel of HLA-DR-typed fibroblasts was used to present antigen to clone G8. This clone was derived from an HLA-DR3,5-positive donor, and was shown to be HLA-DR5 restricted (reference 15 and Table V). Among the eight allogeneic fibroblast lines tested, three of three HLA-DR5(+) lines presented TT antigen to G8, whereas none of four (0/4) HLA-DR5(−) fibroblast lines presented TT antigen to G8 (Table V). Monocytes were available from two of the four HLA-DR5(−) donors whose fibroblasts were tested. In both cases the monocytes failed to present TT to G8. Thus, there was complete concordance with respect to antigen presentation by monocytes versus fibroblasts in the five instances where monocytes and fibroblasts were available from the same donor.

It was imperative to show that IFN-γ-treated HLA-DR5(−) fibroblasts, which failed to present antigen to clone G8, could present antigen to HLA-DR homologous T cell clones. A TT-specific T cell clone (C1) was generated from the HLA-DR2,8 donor shown in Table V. Fibroblasts treated with IFN-γ from this donor were tested for their capacity to present TT antigen to C1. Table VI shows that IFN-γ-treated fibroblasts from this donor were equivalent to monocytes in their capacity to support proliferation of the autologous clone C1 to TT. These results indicate that the failure of HLA-DR5(−) fibroblasts to

Table III. T Cell Clones Are Antigen-specific When Fibroblasts Are the APC

		CPM [³H]thymidine incorporated per culture			
		Antigen			
Clone	APC	—	TT	DT	Monilia
F6	—	117±27	87±16	213±135	99±30
	Monocytes	332±84	15,818±1,219	424±384	416±209
	Fibroblasts IFN-γ	381±51	7,713±1,605	290±172	291±69
G8	—	93±53	124±50	175±137	132±46
	Monocytes	610±233	3,429±816	626±260	703±255
	Fibroblast IFN-γ	180±54	5,932±658	174±23	154±56

Values represent mean±SD of triplicate ±conditions. Culture conditions were as described in Table I. TT and DT were used at 30 μg/ml. Monilia antigen (Hollister Stier) was used at a final dilution of 1:100. In addition, G8 was passed over Sephadex G10 and then treated with OKM1 and Leu M1 plus complement to remove any residual monocytes. [³H]Thymidine incorporation by monocytes was <340 cpm and by fibroblasts <120 cpm in the two experiments.

Table IV. Presentation of Antigen by TT-pulsed IFN-treated Fibroblasts

APC	Antigen	CPM [^{3}H]thymidine incorporated per culture	
		Clone F6	Clone G8
None	—	172±53	151±36
None	Soluble TT	287±161	402±125
EBV B cells	—	399±193	247±48
EBV B cells	Soluble TT	15,479±3,432	8,211±401
Fibroblasts IFN-γ	—	530±185	726±138
Fibroblasts IFN-γ	Soluble TT	10,522±2,309	9,369±886
Fibroblasts IFN-γ	TT pulsed	10,063±797	12,631±2,166
Fibroblasts	TT pulsed	824±146	396±81

Values represent mean±SD of triplicate cultures. Culture conditions were as described in Table I. Fibroblasts were pulsed with antigen by preincubating with TT (50 μg/ml) at 37°C for 20 h, then washed three times with medium before addition of the T cell clones.

present antigen to the DR5-restricted clone G8 (Table V) was not caused by an intrinsic inability of the fibroblasts to present antigen. Taken together, these results suggested that the presentation of antigen by IFN-γ-treated fibroblasts to T cells was MHC restricted.

We next examined the effect of mAb to HLA-DR on antigen presentation by IFN-γ-treated fibroblasts. Table VII shows that addition of monoclonal anti-HLA-DR (LB3.1) to the cultures abolished T cell proliferation. In contrast, monoclonal anti-HLA-A,B (W6/32), monoclonal anti-DC (Leu 10), and an irrelevant mAb (UPC 10) did not inhibit T cell proliferation. The blocking by LB3.1 was not an isotype effect because BBM.1, an antibody reactive with B_2 microglobulin, and of the same immunoglobulin subclass as LB3.1, bound fivefold more to fibroblasts, yet failed to inhibit the proliferation of T cell clones to antigen presented by autologous IFN-γ-treated fibroblasts (data not shown). Furthermore, inhibition of the antigen response by anti-HLA-DR was not simply due to binding of the antibody to the HLA-DR antigen on cloned T cells, because proliferation of the cloned T cells in response to IL-2-containing supernatants was not affected by these antibodies.

The Ia specificity of the inhibition of LB3.1 was confirmed with another mAb to human HLA-DR (ST2-59), which also inhibited T cell proliferation in response to TT presented by autologous IFN-γ-treated fibroblasts, but not in response to IL-2. Fig. 1 depicts the dose dilution curve of ST2-59 on the inhibition of proliferation of G8 and F6. The proliferation of G8 and F6 in response to antigen presented by Ia-positive fibroblasts was inhibited even by low concentrations of ST2-59. In contrast, ST2-59 did not significantly affect the response of these T cell clones to IL-2. These data taken together support the idea that HLA-DR antigens induced by IFN-γ on fibroblasts allow antigen-specific, Ia-restricted proliferation of clones to occur.

Antigen presentation to peripheral blood T cells. The ability of fibroblasts to present TT to autologous resting T cells was examined next. PBMC were depleted of monocytes by adherence to plastic (performed twice), then by passage over Sephadex G10. Table VIII shows that such monocyte-depleted populations of T cells were unable to respond to TT or to concanavalin A without the addition of APC. Autologous-irradiated monocytes or autologous IFN-γ-treated fibroblasts were both able to restore the response of the purified resting T cells to TT. However, the proliferative response of T cells to TT with fibroblasts as the APC was substantially less than that seen with monocytes. These results suggest that IFN-γ fibroblasts were not as efficient as monocytes in presenting soluble antigen to resting T cells.

Discussion

Our experiments show that human dermal fibroblast cultures, pretreated with IFN-γ to induce expression of surface Class II

Table V. MHC Restriction of Fibroblast Presentation of TT Antigen to the HLA-DR5-restricted TT-specific Clone G8

HLA-DR of APC donor	CPM of [^{3}H]thymidine incorporated per culture			
	With fibroblasts		With monocytes	
	—	TT	—	TT
3, 5 (Autologous)	379±49	24,281±1,880	2,949±302	12,531±27
3, 5	388±56	8,855±3,848	676±882	8,683±103
5, 6	414±73	4,033±707	220±52	8,872±866
5, 6	225±45	7,292±501	nd	nd
3, 5	nd	nd	650±330	28,558±1,255
9, —	181±44	170±38	3,377±478	3,975±270
2, 8	215±28	832±423	335±59	1,163±177
7, —	1,458±307	1,020±230	nd	nd
2, —	169±12	245±79	nd	nd
3, —	nd	nd	223±52	408±147
3, 4	nd	nd	262±107	322±17
1, 7	nd	nd	264±24	330±46

nd, Not done. Monocytes or fibroblasts were not available from these donors. Values represent mean±SD of triplicate cultures. Culture conditions were as described in Table I.

Table VI. Non-HLA-DR5 Fibroblasts Present TT to Autologous Antigen-specific T Cell Clones

APC	Antigen	CPM of [^{3}H]thymidine incorporated per culture ±SD Clone C1
None	TT	343±120
Monocytes	—	1,295±854
Monocytes	TT	72,100±5,678
Fibroblasts-IFN-γ	—	2,408±148
Fibroblasts-IFN-γ	TT	67,640±6,323

Values represent mean cpm±SD for triplicate cultures. Culture conditions and pretreatment of fibroblasts with IFN-γ were as in Table I. Fibroblasts-IFN-γ, IFN-γ-treated fibroblasts. Both Clone C1 and fibroblasts were derived from the HLA-DR2, 8 donor of Table V.

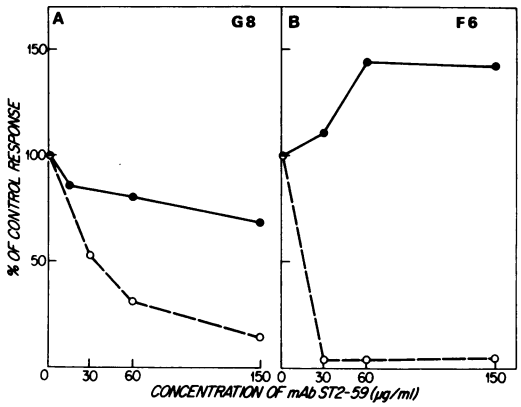

Figure 1. Dose dilution curve of the inhibition of proliferation of the TT-specific clone G8 (*A*) and clone F6 (*B*) by the monoclonal anti-human Ia, ST2-59. Autologous IFN-γ-treated fibroblasts were used to present TT antigen (○) and 20% IL-2 containing supernatants were used as controls (●). 100% of the G8 proliferative response equalled 19,925 cpm. 100% of the F6 proliferative response equalled 7,713 cpm.

MHC antigens, are capable of presenting soluble protein antigen to T lymphocytes in an MHC-restricted manner.

Several lines of evidence make it unlikely that some nonfibroblast cell type in our culture was responsible for antigen presentation. First, it is unlikely that the fibroblast cultures were contaminated by classical APC, since these lines have been propagated at least through 20 population doublings (minimum of 10 subcultures at 1:4 split ratios). The hypothetical contaminating cells in the fibroblast cultures would have to be capable of extensive propagation (i.e., not a typical macrophage), and would require IFN-γ pretreatment to be able to present antigen, which implies that it would be Ia-negative until induction; i.e., not a typical dendritic cell. Although our fibroblasts are not cloned, they were morphologically homogeneous and uniformly Ia inducible by IFN-γ.

Second, it is also unlikely that the T cell clones were contaminated to any significant extent with APC, which were derived from the irradiated PBMC that were added periodically to maintain these clones. AC activity of such feeder cells is unlikely to have remained as long as 14–28 d after irradiation, the time at which these clones were used. Such clones indeed could not respond to TT in the absence of additional accessory cells (Table I). Furthermore, passage of the clones over Sephadex G10 followed by lysis with anti-OKM1 and Leu M1 plus complement did not affect their ability to respond to antigen presented by IFN-γ fibroblasts (Table II). If, despite all these efforts, a contaminating accessory cell remained in our T cell clone preparations, then, at the very least, MHC compatible Ia(+) fibroblasts would be required for that residual accessory cell to express its function (Table V). Thus, the fact that antigen presentation to T cell clones by fibroblasts appeared MHC restricted, argues strongly that the APC was derived from the fibroblast cultures, and not from the T cells.

Our present experiments characterize antigen presentation by human dermal fibroblasts. First, Ia-positive fibroblasts, but not Ia-negative fibroblasts, stimulate antigen-specific T cell proliferation in cloned T cells (Tables I and IV). Second, antigen-pulsed Ia-positive fibroblasts effectively induce T cell proliferation, which suggests that these cells have the intrinsic capacity to take up and present antigen (Table IV). Third, antigen presentation by fibroblasts to cloned T cells appears to involve fibroblast DR-antigens, as evidenced both by MHC restriction and by inhibition with mAb to HLA-DR antigens (Tables V and VII, Fig. 1). Although our data are limited to three T cell clones, they are in accord with knowledge of the role of DR and other Class II MHC antigens established unambigously for T cell activation by monocytes and other AC types. Thus, fibroblasts behave in a manner completely analogous to classical APC, and fibroblasts must be added to

Table VII. Monoclonal Anti-HLA-DR Inhibits Proliferation of F6 Induced by Fibroblasts

IFN-treated fibroblasts	Stimulus	CPM of [^{3}H]thymidine incorporated per culture in the presence of: Media	LB3.1 (anti-HLA-DR)	W6/32 (anti-HLA-A,B)	Leu 10 (anti-DC)	UPC10 (control)
+	—	288±120	211±12	354±129	306±4	343±182
+	TT	6,567±279	891±345	6,804±1,295	6,393±770	7,107±1,513
—	IL-2	8,120±475	6,864±1,076	7,297±1,920	6,312±460	8,828±1,575

Values represent mean±SD. Culture conditions and pretreatment of fibroblasts are as in Table I. mAb (ascites) was added at 1:100 final dilution, and was present throughout the culture period. IL-2 containing supernatants were used at 1:4 dilution. Similar results were obtained with clone G8.

Table VIII. Fibroblasts Present Antigen to Autologous Macrophage-depleted PBMC*

APC	Stimulus	CPM of [^{3}H]thymidine incorporated per culture ±SD	
		Exp. 1	Exp. 2
—	—	1,781±1,361	366±319
—	Concanavalin A	435±250	273±133
—	TT	1,682±911	594±148
Monocytes	—	970±559	118±24
Monocytes	TT	97,712±7,339	44,132±10,092
Fibroblasts IFN-γ‡	—	562±89	758±349
Fibroblasts IFN-γ‡	TT	20,614±5,652	6,837±2,884

10^5 monocyte-depleted PBMC were cultured for 6 d, alone, or with 2×10^5 irradiated monocytes, or with IFN-γ-treated fibroblasts. TT, 30 μg/ml, was added to the indicated cultures. Values represent mean of triplicate cultures ±SD. * PBMC depleted of adherent cells by adherence to plastic overnight and passage over Sephadex G10 columns. ‡ Interferon treated.

the list of other cell types that are capable of antigen presentation.

Normal fibroblasts have not been previously thought capable of antigen presentation. Although Katz and Unanue (23) reported that mouse embryo fibroblasts pulsed with DNP-KLH caused the development of a significant anti-DNP plaque-forming cell response in whole spleen cell cultures, the spleen cells used were not depleted of macrophages, and it remained unclear which APC stimulated the T lymphocytes. Lipsky et al. (24) showed that guinea pig kidney fibroblasts could reconstitute the proliferative response of purified T cells to phytohemagglutinin, but not to the tuberculin antigen. Similar results were reported by Raff (25) using mouse embryo fibroblasts. Thus, fibroblasts, which normally do not express Ia antigens, failed to present protein antigens to T cells. This idea was consistent with the dictum that Ia expression is necessary for protein antigen presentation. Recently, Hood and coworkers (26) have transferred genes for Ia antigens into mouse fibroblasts. Expression of these genes allowed such fibroblasts to stimulate antigen-specific T cell hybridomas to secrete IL-2. Our experiments have used a physiologic stimulus, i.e., IFN-γ, to induce Ia expression on fibroblasts, which then presented antigen and induced proliferation of T cells.

In vivo fibroblasts do not normally display surface Ia antigens. As in the case of macrophages (27), modulation of Ia expression on fibroblasts may be a mechanism by which their antigen presenting activity is regulated. Positive modulation of Ia expression in fibroblasts with IFN-γ secreted by T cells (28) may serve to amplify in vivo immune responses, and to allow the ubiquitous fibroblasts to participate in local immune responses. Sustained Ia expression by dermal fibroblasts in tissues infiltrated by activated T cells may perpetuate the immune response and produce chronic inflamation and tissue injury.

We do not suggest, however, that fibroblasts play a primary role in antigen presentation in vivo. Although fibroblasts can support proliferation of cloned T cells to an extent similar to that observed with monocytes, fibroblasts appear to function less well than monocytes in presenting antigen to resting T cells (Table VIII). In this respect, we have recently shown that although EBV-transformed B cells serve as excellent accessory cells for antigen-specific T cell clones, they fail to support antigen-specific proliferation of highly purified resting T cells (29). Thus, it appears that the requirements to activate a resting T cell may be best provided by a cell of monocyte/macrophage lineage, but the requirements for activation of cloned T cells can be easily met by multiple Ia(+) cell types.

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